

A FURTHER NOTE ON THE NITROGEN METABOLISM OF STEREUM GAUSAPATUM FRIES¹

J. ARTHUR HERRICK AND CONST. J. ALEXOPOULOS,
Kent State University

In an earlier paper by the senior author (Herrick, 1940), the results of a study of the ability of *S. gausapatum* Fries to utilize nitrogen from various sources were reported. The general conclusion was that this fungus could not obtain nitrogen from inorganic compounds. More recent investigations, which constitute the basis for this paper, make necessary a revision of the earlier conclusion.

The earlier data show that growth on synthetic liquid media containing any one of several inorganic nitrogen compounds or asparagine, as the only source of nitrogen, is not significantly greater than that on a nitrogen free medium. When an equal amount of nitrogen is provided in the form of peptone a luxuriant mycelium is produced.

In the course of some other studies by the authors, it was noticed that the growth of this organism is greatly stimulated by minute amounts of thiamin. Like stimulation of other organisms has been reported in the literature (Addinall, 1940). Following these observations, a careful investigation was undertaken and is reported in the following pages.

EXPERIMENTAL STUDIES

In order to determine the growth promoting effect of thiamin on *S. gausapatum* the following experiment was performed. A synthetic liquid culture medium containing 4.5 gms. of NH_4NO_3 per liter as the only source of nitrogen was prepared. The basic formula for this medium has been previously published (Herrick, 1940). This medium was divided into 50 cc. portions in 200 cc. Erlenmeyer flasks. Thiamin (Merck's Betabion) was dissolved in distilled water, and by dilution a series of concentrations prepared. By the addition of 1 cc. of the appropriate solution to each flask, respectively, culture media were prepared having thiamin content ranging from 10^{-3} to 10^{-10} gms. per flask, respectively. Checks were prepared by the addition of 1 cc. of distilled water, instead of thiamin, to each of several flasks. These flasks were then plugged and autoclaved for 15 minutes at 15 lbs. pressure. Immediately after cooling, each flask was inoculated with a 4 mm. disk cut from a corn-meal-agar petri dish culture of a single spore isolate² of *S. gausapatum*. Twenty-eight days after inoculation five cultures from each medium were filtered through each of several previously dried and weighed filter papers. The papers, plus fungous material, were dried at 103° C. for 24 hours and weighed.

The dry weights of fungous mycelium produced on each of the several media are presented in Table 1.

The data presented in Table 1 prove conclusively that this fungus can obtain significant amounts of nitrogen from at least one inorganic compound, and further, the amount of growth is, within certain limits, roughly proportional to the amount of thiamin present. This does not allow us to generalize about the use of nitrogen compounds and so another experiment was devised as follows: Eight liters of a nitrogen-free medium were prepared according to the previously reported formula (Herrick, 1940). This was autoclaved and filtered to dispose of the precipitate.

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²The isolate used was "S-12," described in a previous publication (Herrick, 1939).

The original volume was then restored by the addition of distilled water. To one liter portions of this nitrogen-free medium, nitrogen-containing compounds were added so that each contained the same amount of nitrogen as was present in the above experiment using NH_4NO_3 . The exact amounts added are shown in the accompanying table (Table 2). Each of these several media was divided into two equal lots. To one portion thiamin was added at the rate of 10^{-4} gms. per 50 cc. The corresponding media without the thiamin served as checks and were the same as those reported in the earlier paper (Herrick, 1940). The media were

TABLE I
THE EFFECT OF THIAMIN ON THE GROWTH OF *S. gausapatum* ON SYNTHETIC LIQUID MEDIA CONTAINING NH_4NO_3 AS THE ONLY SOURCE OF NITROGEN.

Thiamin Content per Flask of 50 cc. of Medium. (Expressed in Grams)	Dry Weight of the Mycelium Produced in Five Flask-Cultures in 28 Days. (Expressed in Grams)
0.001	0.278
0.0001	0.315
0.00001	0.268
0.000001	0.265
0.0000001	0.117
0.00000001	0.157
0.000000001	0.052
0.0000000001	0.045
No Thiamin	0.025
Uninoculated Check	0.011 (Due to precipitate)

TABLE II
THE GROWTH OF *Stereum gausapatum*, USING VARIOUS COMPOUNDS AS A SOURCE OF NITROGEN, WITH AND WITHOUT THIAMIN.

Source of Nitrogen	Amount of Nitrogen Compound per Liter of Medium	Dry Weight of Mycelium Produced in 5 Flask-Cultures in 28 Days. No Thiamin	Dry Weight of Mycelium Produced in 5 Flask-Cultures in 28 Days. Thiamin added
Peptone.....	10.00 gms.	0.792 gms.	1.439 gms.
Asparagine.....	7.25 "	0.033 "	0.853 "
NH_4NO_3	4.50 "	0.015 "	0.160 "
NH_4Cl	6.75 "	0.009 "	0.270 "
$(\text{NH}_4)_2\text{SO}_4$	7.75 "	0.017 "	0.255 "
KNO_3	11.50 "	0.014 "	0.014 "
NaNO_3	9.75 "	0.008 "	0.016 "
No Nitrogen.....		0.013 "	0.011 "

divided into 50 cc. portions in Erlenmeyer flasks, plugged and autoclaved for 15 minutes at 15 lbs. pressure. After cooling, the flasks were uniformly inoculated with 2 mm. disks cut from a vigorously growing corn-meal-agar petri dish culture of the isolate used previously. Twenty-eight days after inoculation five flask-cultures of each medium were filtered through each of several previously dried and weighed filter papers, respectively, and the dry weight of the fungus determined by the method described above.

An examination of the data presented in Table 2 leads to an expansion of the previous conclusion, i. e., that this fungus is able to obtain appreciable quantities of nitrogen, not only from NH_4NO_3 but from NH_4 —containing compounds and from asparagine. The $-\text{NO}_3$ ion appears to be of no value to this organism under the conditions of this experiment. In this connection it should be noted that the mycelial growth with NH_4NO_3 is equal to roughly half that produced on media containing NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$. This is significant since each medium contains the same amount of nitrogen and in the case of NH_4NO_3 about one-half of the nitro-

gen is in the form of $-\text{NO}_3$. The nitrogen of asparagine is apparently more readily obtained than that of the $-\text{NH}_4$ ion. The ability of thiamin to stimulate growth on peptone medium is of no special interest in the present study but serves as a basis of comparison for the other data.

That K^- and Na^- ions are sometimes toxic is a well known fact. Two lines of evidence indicate that the lack of growth on the KNO_3 and NaNO_3 media was not due to such toxicity. First, the addition of peptone to such media enables it to support a heavy growth of mycelium (Herrick, 1940). As a further check, a culture medium was prepared in which there was a balance of antagonistic ions (Miller, 1938, p. 269). The medium had the following composition:

H_2O	1000.0 gms.
Sucrose.....	50.0 gms.
KH_2PO_4	5.0 gms.
MgSO_4	2.5 gms.
FeCl_2	0.2 gms.
NaNO_3	8.7 gms. (making 0.1 M solution)
KNO_3	22 cc. of a 0.1 M solution
CaCl_2	10 cc. of a 0.1 M Solution

This medium was divided into four lots and altered as follows:

1. Nothing added.
2. 10^{-4} gms. of thiamin added per 50 cc.
3. 1% peptone added.
4. 1% peptone plus 10^{-4} gms. thiamin per 50 cc. added.

Five 200 cc. Erlenmeyer flasks, each containing 50 cc. of solution, were prepared with each medium. These were inoculated, allowed to incubate for 28 days, and the dry weight of the mycelium was then obtained. The growth in the two sets containing peptone approximately duplicated the earlier results on peptone-containing medium (Table 2). The growth in the medium containing only $-\text{NO}_3$, or $-\text{NO}_3$ plus thiamin, as the only source of nitrogen was quite like that normally obtained on nitrogen-free medium. It is therefore concluded that the lack of growth on media containing $-\text{NO}_3$ ions as the only source of nitrogen is not due to any toxic effect of Na^- or K^- ions but to the inability of *S. gausapatum* to use the $-\text{NO}_3$ ions.

SUMMARY AND CONCLUSIONS

The ability of *Stereum gausapatum* to obtain nitrogen from various sources has been re-investigated.

As reported earlier (Herrick, 1940), synthetic media containing inorganic salts or asparagine as the only source of nitrogen support little or no growth. Peptone-containing medium produces a heavy mycelium. The growth on media containing peptone, asparagine or $-\text{NH}_4$ ions, as the only source of nitrogen, is greatly increased by the addition of minute amounts of thiamin. In the case of NH_4NO_3 the growth was found to be roughly proportional to the thiamin content of the medium. The growth on media containing only $-\text{NO}_3$ ions as a source of nitrogen was negligible, even though thiamin was present. The lack of growth in such cases was shown not to be due to any toxic effect of cations. This fungus is therefore able to use asparagine and ammonium salts, in addition to peptone, as sources of nitrogen, the thiamin content acting as a limiting factor.

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